

All  
cont'd

sequence included genbank numbers BE311583, BE296956, BE301939, AI677957, AI913163, AW60648, AI653716, BE763461, and AB037816.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 118, at the end of the application.

REMARKS

The amendment to the paragraph beginning at page 24, line 16 amends the "DOCK" motif peptide sequences in line 20 and the motif labels in line 19. This is supported by the two motifs found in FIG. 3B. These amino acid motifs presently found on line 20, i.e., (P+EXAI+XM) and (LXMXL+GXVXXXVNXG), which are presented in FIG. 3B, boxes E and F, on the two lines labeled "CONSENSUS", appear as:

P+E AI+ M

+

and

L M L+G V VN G

L I,

respectively. The "+" symbol and the letters "L" and "I" on the second "CONSENSUS" line indicate optional amino acid variants at these indicated positions occur in their homology comparison. Therefore, the terminal "M" residue in the first motif (motif E) has been amended to a "+" symbol to reflect these variants. The two positions in the second motif (motif F) have also indicated these optional amino acid variants by the "M/L" and "V/I" designations. The Sequence Listing has incorporated the indicated optional variants into their respective positions.

The amendment includes amending the letters to which those highly conserved non-tyrosine containing regions refer. The first amino acid motif is presented in FIG. 3B, page 1 of 2, where the last region boxed is labeled region E. This region is followed by a boxed region labeled "F" in FIG. 3B, page 2 of 2, instead of region "G" as designated on page 24, line 19.

This amendment further corrects the number of amino acid residues stated to be separating these two motif sequences in line 20. Although the number of amino acid residues between boxes E and F is stated to be nine on line 20 of the Specification (illustrated in FIG 3B as eight residues on page 1 of 2 and one residue on page 2 of 2), FIG. 3B does not illustrate the entire contiguous sequence of amino acids in the CLASP protein homologs. The actual number of amino acid residues appearing in the CLASP protein sequences corresponding to these motifs appears in FIG. 3A, where all contiguous residues are represented. In FIG 3A, page 5 of 5, the regions of homology surrounding the box labeled "Coiled-Coil 1" contain the corresponding regions labeled motifs E and F from FIG. 3B. These motifs extend from the "P" residue one amino acid to the left of the boxed "Coiled-Coil 1" in the top line of the homology groupings to seven amino acid residues into the second line of homology groupings, where the conserved "...VNXXG" terminal portion of motif F occurs. By comparison of the E and F motifs with the sequences in FIG 3A, it can be seen that the actual number of residues separating these motifs is actually 20. Thus, no new matter has been introduced by the amendments to this paragraph.

Applicants request entry of this amendment in adherence with 37 C.F.R. §§1.821 to 1.825. This amendment is accompanied by a floppy disk containing the above named sequences, SEQ ID NOS:1-115, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

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PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Andrew T. Serafini". The signature is fluid and cursive, with a large, stylized initial "A".

Andrew T. Serafini  
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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 6 of page 6 has been amended as follows:

**Figure 1.** Preliminary CLASP-7 cDNA sequence (SEQ ID NO:59; amino acid sequence = SEQ ID NO:60). Notable protein motifs are labeled above the nucleotide sequence.

Paragraph beginning at line 21 of page 6 has been amended as follows:

**Figure 3. A.** Amino acid sequence of human and rat CLASP proteins. Sequences were aligned using ClustalW. One letter amino acid abbreviation used. Protein motifs are found within the labeled boxes. -A “-” indicates gaps that are placed to acquire a best overall alignment. Other abbreviations: “HC2A” Human CLASP-2 sequence (SEQ ID NO:9), “KIAA” KIAA1058 sequence (SEQ ID NO:10) (Genbank Accession No. AB028981), -“rat” TRG gene (SEQ ID NO:11) (Genbank Accession No. X68101), “HC4” -Human CLASP-4 sequence (SEQ ID NO:12), “HC1” Human CLASP-1 sequence (SEQ ID NO:13), “HC3” Human CLASP-3 sequence (SEQ ID NO:14), “HC5” Human CLASP-5 sequence (SEQ ID NO:15). **B.** Alignment of DOCK motifs found within the human CLASPs (SEQ ID NOS:16-20, 24, 25, 27-31, 35, 37-43, 47 and 49-55) and rat TRG (SEQ ID NOS:26, 36 and 48) and compared to canonical DOCK motifs (SEQ ID NOS:21-23, 32-34, 44-46 and 56-58). Consensus amino acids found within all DOCK motifs are also indicated.

Paragraph beginning at line 25 of page 7 has been amended as follows:

**Figure 6.** Sequence of human CLASP-7 exons and introns. **A)** Sequence of human CLASP-7 exons and intron borders (SEQ ID NOS:61-103). Stretches of noncontiguous genomic sequence from the Human Genome Project (GENBANK entry gi7711509) were aligned using the human CLASP-7 cDNA as a template and Sequencher sequence analysis software. Due to the incompleteness of the Human Genome Project, only partial genomic sequence from human CLASP-7 was obtained. 43 exons representing approximately -the 5' 70% of the human CLASP-7 cDNA sequence are presented in predicted 5' to 3' order. Exon sequences are underlined and are flanked by intron sequence. This exon/intron map could only have been produced having the isolated human CLASP-7 cDNA. Nucleotide numbers for the exons refer to the intron/exon sequences listed in FIG. 6A within the noncontiguous gi7711509 sequence. **-B)** Putative promoter region (SEQ ID NO:104) upstream of the 1<sup>st</sup> identified exon of human CLASP-7. This sequence represents nucleotide numbers 61938 to 63888 of GENBANK entry gi7711509 presented in the 5' to 3' direction. The 5' terminus of this sequence is also the end of the contiguous piece of DNA listed in gi7711509. Underlined sequence represents the expressed first exon.

Paragraph beginning at line 9 of page 8 has been amended as follows:

**Figure 7.** Amino acid alignment and comparison between the human (h) CLASP family members (SEQ ID NOS:105-110). Amino acid sequences were aligned using ClustalW. The alignment is presented in order of their greatest pairwise similarity scores. Single letter amino acid abbreviations are used. Astericks indicate complete identity, while colons and periods indicate sequence similarity among CLASP family members. Dashes indicate gaps inserted in the amino acid sequence to facilitate alignment. Labelled boxes are domains with similarity to known protein motifs;

unlabelled boxes represent regions of similarity between all CLASPs and may represent CLASP-specific domains.

Paragraph beginning at line 14 of page 21 has been amended (reformatted from indented form):

The human CLASP-7 sequence presented in FIG. 5 encodes one potential start site for translation. The predicted methionine appears at nucleotide +1 (ATG). It is an acceptable consensus sequence for a translational start (A/GxxATGG; Kozak, M. 1996, *Mamm. Genome* 7(8): 563-74). Due to the lack of in-frame stop codons upstream of the predicted initiator methionine in FIG. 5, a second possibility for a translational start is that the cDNA listed in FIG. 5 is incomplete and another methionine is encoded in frame and upstream of the sequence shown in FIG 5. **Extracellular Domain**

Paragraph beginning at line 15 of page 21 has been amended as follows:

The CLASP-7 extracellular domain is characterized by one cadherin EC-like motif (Pigott, R. and Power, C., 1993, *The Adhesion Molecule Factbook*. Academic Press, pg. 6; Jackson, R. M. and Russell, R. B., 2000, *J. Mol. Biol.* 296: 325-34). Several highly conserved cysteines are found in the extracellular domain, as well as various glycosylation signals. Through its extracellular domains, CLASP-7 may interact with ligands in a homotypic and/or heterotypic manner to establish the immunological synapse in conjunction with molecules such as TCR, MHC class I, MHC class II, CD3 complex and accessory molecules such as CD4, CD3, ICAM-1, LFA-1, and others. Many cadherins contain a pro-domain of approximately 50 to 150 amino acids that is removed before localization to the plasma membrane. This cleavage is presumed to be carried out by Furin (Posthaus, H. *et al.*, 1998, *FEBS Let* 438: 306-10) at a consensus sequence of

RKQR (SEQ ID NO:3). Furin is a protease that is at least partially responsible for the maturation of certain cadherins. CLASP-7 contains the amino acid sequence RKLR (SEQ ID NO:4) encoded by nucleotides 2866 to 2877 shown in FIG. 1. By homology, this region is around 956 amino acids into the predicted protein start site for hCLASP-7 indicated in FIG. 5.

Paragraph (Table 1) beginning at line 16 of page 23 has been amended as follows:

**Table 1**  
**CLASP-7 ITAM Motifs**

Motif No.	Sequence Motif	<u>SEQ ID NO:</u>
1	YXXV-X <sub>2</sub> -YXXH	<u>5</u>
2	YXXI-X <sub>5</sub> -YXXT	<u>6</u>

Paragraph beginning at line 16 of page 24 has been amended as follows:

CLASP-7 polypeptides contain a new "DOCK" motif, not previously described in the scientific literature. The CLASP DOCK motif includes a series of five tyrosines surrounded by conserved sequences in regions A, B, C, D, and G (see FIG. 3B). There are also two highly conserved non-tyrosine containing regions (E and ~~F-G~~) separated by 20 ~~nine~~ amino acids (P+EXAI+X+; SEQ ID NO:7) (~~P+EXAI+XM~~) and (LX(M/L)XL+GX(V/I)XXXVNXG; SEQ ID NO:8) (~~LXMXL+GXVXXXVNXG~~) (where X is any amino acid).

Paragraph beginning at line 12 of page 53 has been amended as follows:

In one embodiment, the antisense sequence is complementary to relatively accessible sequences of the CLASP-7 mRNA (*e.g.*, relatively devoid of secondary structure). This can be determined by analyzing predicted RNA secondary structures using, for example, the MFOLD program (Genetics Computer Group, Madison WI) and

testing in vitro or in vivo as is known in the art. Another useful method for identifying effective antisense compositions uses combinatorial arrays of oligonucleotides (see, *e.g.*, Milner *et al.*, 1997, Nature Biotechnology 15: 537). Examples of oligonucleotides that can be tested in cells for antisense suppression of CLASP-7 function are those capable of hybridizing to (*i.e.*, substantially complementary to) the CLASP-7 at the following positions:

Oligo	Sequence 5' - 3'	length	notes/comments
1	CCCCCAAGACGCTCTCCCG GGCTTCTGAAAG ( <u>SEQ ID NO:111</u> )	31-mer	spans nucleotides 2-38 of the sequence of FIG. 1 (nucleotides 4213-4243 of the cDNA sequence shown in FIG. 5)
2	CCGCGTGCACCATGCACTG GGCGGCCTCGGC ( <u>SEQ ID NO:112</u> )	31-mer	spans nucleotides 629-659 of the sequence of FIG. 1 (nucleotides 4840-4870 of the cDNA sequence shown in FIG. 5), and is complementary to the region encoding the transmembrane domain
3	GGCCAGCTCCCGTGTCTTC TTCTGCATGTCCTCG ( <u>SEQ ID NO:113</u> )	34-mer	spans nucleotides 1507-1540 of the sequence of FIG. 1 (nucleotides 5718-5751 of the cDNA sequence shown in FIG. 5), and is complementary to the region encoding the first coiled coil domain

Paragraph beginning at line 10 of page 54 has been amended as follows:

The antisense nucleic acids (DNA, RNA, modified, analogues, and the like) can be made using any suitable method for producing a nucleic acid, such as the chemical synthesis and recombinant methods disclosed herein. In one embodiment, for example, antisense RNA molecules of the invention can be prepared by de novo chemical synthesis or by cloning. For example, an antisense RNA that hybridizes to CLASP-7 mRNA can be made by inserting (ligating) an CLASP-7 DNA sequence (*e.g.*, SEQ ID NO:1 ~~SEQUENCE ID No: 1~~, or fragment thereof) in reverse orientation operably linked to a promoter in a vector (*e.g.*, plasmid). Provided that the promoter and, preferably



termination and polyadenylation signals, are properly positioned, the strand of the inserted sequence corresponding to the noncoding strand will be transcribed and act as an antisense oligonucleotide of the invention. The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter or enhancer) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

Paragraph beginning at line 12 of page 107 has been amended as follows:

To obtain additional 5' CLASP-7 sequence, portions of the cDNA were compared to the NCBI database by BLAST. A genomic clone (Genbank identifier: gi7711509 comprising random, shotgun genomic sequence was identified. Using TFASTX (Pearson and Lipman, PNAS (1988) 85:2444-2448), the amino-terminal sequence of human CLASP-7 as well as amino terminal portions of the human CLASP-1, CLASP-4, and CLASP-2 genes were compared to 6 frame translations of gi7711509 . Areas of gi7711509 that encoded amino acids with high similarity to CLASP-7 or amino terminal portions of human CLASP-1, CLASP-2 or CLASP-4 were used to design CLASP-7-specific oligonucleotides for RT-PCR (reverse transcriptase polymerase chain reaction according to manufacturers instructions: Reverse transcriptase Gibco/BRL, Taq Polymerase from Sigma). Using sense oligonucleotides such as C7gS23 (5' CTGGACTTTGAGGATGTAC (SEQ ID NO:114); nucleotides 160-178 of FIG. 5) and antisense oligonucleotides such as C7AS16 (AGGGTGAAGAATTTGTCCAGG (SEQ ID NO:115); reverse complement of nucleotides 2169-2189 of FIG. 5A) an RT-PCR product of approximately 2 kb was generated, sequenced (dideoxynucleotide termination sequencing, Beckman Coulter CEQ2000) along with other hCLASP-7 RT products and shown to be additional human CLASP-7 5' sequence. Many RT-PCR products isolated in this region were unable to be propagated in bacteria suggesting either a toxic effect on bacteria at the DNA level or the presence of a system in bacteria for selecting against

these sequences. Additional 5' and confirmatory sequence was obtained from Genbank EST and human genomic DNA sequence. ESTs and sequences aside from the genomic clone listed above that were used in generating the complete human CLASP-7 coding sequence included genbank numbers BE311583, BE296956, BE301939, AI677957, AI913163, AW60648, AI653716, BE763461, and AB037816.